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Presence of tumor-specific cytolytic T cells in human primary breast carcinoma: consequences for immunotherapy.

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Immunotherapy through stimulatory antibodies targeting the CTLA-4 or PD-1 pathways has a clear clinical efficacy in a fraction of patients with various cancers. It is likely that the main immune effectors of these therapies are $CD8^+$ cytolytic T lymphocytes (CTL) recognizing tumor-specific antigens. The antigenicity of human tumors has been demonstrated with studies conducted mostly on melanomas. However the genetic mechanisms leading to antigenicity, notably point mutations in the tumor cells, apply to all cancer types. Thus primary breast carcinoma cells do certainly bear tumor-specific antigens, even though the extent of this antigenicity is unknown.

Most melanomas, which are highly antigenic tumors, are also immunogenic, i.e. they stimulate spontaneous anti-tumor CTL responses. This immunogenicity, of which the presence of tumor-infiltrating T cells (TILs) is probably a surrogate marker, might be a predictive marker for clinical benefit to immunostimulatory antibodies. Whether primary breast carcinomas are immunogenic is not known, mainly due the absence of autologous tumor cell lines to analyze patients' T cells. However even in the absence of T-cell aimed immunotherapy the amounts of TILs have been positively correlated with patients' survival.

Here we wished to obtain evidence for the presence of tumor-specific $CD8^+$ T cells in TILs from primary breast carcinomas. From each tumor we isolated TILs and derived a random set of ±100 $CD8^+$ clones maintained in culture by stimulation with anti-CD3 antibodies, thus irrespective of their antigenic specificity. We screened these clones for recognition of tumor-specific antigens present on the autologous tumor. In the absence of autologous tumor lines we restricted our analysis to mutated antigens selected on the basis of tumor exome sequencing and gene expression profiling. Indels and non-synonymous base substitutions were selected to synthesize candidate mutated peptides.

Thus far we have analyzed two hormone receptor-positive HER2-negative primary carcinomas. For one patient we screened 144 T cell clones for recognition of 40 candidate mutated peptides, without any positive result. For the other patient, 6 out of 98 T cell clones recognized 4 out of 119 candidate mutated peptides. Two peptides were recognized by two different T cell clones, i.e. with different T cell receptor sequences. These 4 'antigenic' mutations appear to be passenger, i.e. the four genes have a low published mutation frequency.

We conclude that some human primary breast carcinomas are immunogenic, as one tumor contained at least 6% of tumor-specific T cells among the $CD8^+$ TILs. It suggests that the corresponding patient could benefit from the currently used immunostimulatory antibodies. More work is required to understand the reasons for the

negative results in the first patient. We are pursuing the work on 2 HER2-positive and 2 triple-negative tumors, in which TILs are better correlated with prognosis. Our results warrant more investigations on the activation or inhibition of tumor-specific T cells at early stages of human breast cancer development.